PROTEIN DEGRADATION IN ADRENAL CELLS IN CULTURE IS INHIBITED BY ACTH AND CYCLIC AMP

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1. Introduction

In vivo trophic effects of adrenocorticotropic hormone (ACTH) are well known [1]. Most authors have found that ACTH treatment in vitro stimulates total protein synthesis [2–4]. But the mechanism of this action is unknown. We recently showed that in a functional cloned mouse adrenal cell line (Y_1) ACTH and all compounds able to increase cyclic adenosine 3′5′-monophosphate (cAMP) induce a dramatic increase of the incorporation of radioactive leucine into a small peptide (mol. wt 3500) [5]. But this increase cannot explain the enhanced rate of total protein synthesis produced by ACTH. Likewise the stimulatory effect of ACTH on other specific adrenal proteins [6,7] is not sufficient to account for its effect on total adrenal protein synthesis.

One could therefore postulate that ACTH action on protein synthesis involves alterations in the rate of degradation of adrenal proteins. The present paper reports for the first time that corticotropin 1–24 tetracosapeptide ($ACTH_{1-24}$), two of its analogues: corticotropin-(11–24)-tetradecapeptide ($ACTH_{11-24}$) and (9-tryptophan(*o*-nitrophenyl sulfenyl))-corticotropin (1–24)-tetracosapeptide ($NPS\text{-}ACTH_{1-24}$) and dibutyryl cyclic adenosine 3′5′-monophosphate (dbcAMP) are able to inhibit in vitro adrenocortical protein degradation.

2. Materials and methods

$ACTH_{1-24}$ was provided by Ciba. $NPS\text{-}ACTH_{1-24}$ and $ACTH_{11-24}$ were a gift from Drs Rittel and Desaulles (Ciba-Geigy AG, Basel, Switserland). [^{3}H]-Leucine (spec. act. 25–35 Ci/mmol) was obtained from Saclay, France.

Functional cloned mouse adrenal tumor cells (Y_1) developed in the laboratory of G. Sato [8] were purchased from the American Culture Cell Repository (Rockville, MD). They were grown in Ham's F-10 medium supplemented with 10% heat-inactivated horse serum and 2.5% heat-inactivated fetal calf serum (FCS).

Protein degradation was measured in the cells 2 days after seeding the same amount of cells (5×10^6 cells) in 25 cm^2 Falcon flasks. They were first incubated for 15 h with 15 μCi/ml [^{3}H]leucine, 0.1 mM, in Ham's F-10 supplemented with 0.2% FCS. At the end of the labeling period the cells were rinsed twice with 3 ml Ham's F-10 supplemented with 0.2% FCS and 5 mM leucine, and incubated 3 h in this medium. At the end of this chase period they were incubated in 3.3 ml of the same medium in the presence or absence of hormone or dbcAMP. In these conditions of serum deprivation it has been shown that these cells are synchronized in G_1 [9].

Repeated samples of medium (100 μl) were taken from the same flask during the experiment, precipitated by equal vol. 20% trichloroacetic acid (TCA) in the presence of 10 μl 10 mg/ml bovine serum albumin. After centrifugation, 180 μl supernatant were counted in 5 ml scintillation fluid (8.25 g Scintimix, 500 ml Triton X-100 in 1 litre toluene). Each experimental point was run in triplicate, and each experiment was performed at least 3 times. Measurement of the trichloroacetic acid-soluble radioactivity in the medium of cultured cells has been shown to

give a good estimation of protein degradation in cultured cells, especially in turnover experiments [10]. Nevertheless at the end of the sampling period, the cells were rinsed and solubilized as described [5,6] and the TCA-soluble radioactivity in the cell was measured. 20α-hydroxypregn-4-ene-3-one (20αOH progesterone) was measured by a radioimmunoassay (antibodies of E. G. Abraham: S1556#5) following a method described [11] except that the steroid was measured directly in the medium without prior fractionation in a celite column.

3. Results and discussion

After a labeling period of 15 h and a chase period of 3 h the adrenal cells were incubated with two different concentrations of ACTH, 10^{-8} M and 10^{-6} M, in the presence of 5 mM leucine during 27 h (fig.1). After 40 min incubation, the TCA-soluble radioactivity of the ACTH-treated cells represents 98% of the controls. But after 6 h incubation, the inhibition of the degradation is obvious since the TCA-soluble radioactivity in the medium of the ACTH-treated cells represents 80% control. After 20 h incubation, the inhibition induced by the hormone reaches a plateau. The TCA-soluble radioactivity in the medium of the treated cells represents 75% control. Other polypeptide hormones, like human chorionic gonadotrophin (hCG CR 115 provided by NIH, at a concentration of 10^{-8} M), have no effects.

The same experiment was undertaken in the presence of dbcAMP 1 mM, and 20αOH progesterone 100 ng/ml which is the main steroid produced by this cell line. Figure 2 shows that dbcAMP but not 20αOH progesterone has an inhibitory effect on protein degradation. In the same experiment the TCA-soluble radioactivity of the solubilized cells was measured at the end of the 24 h incubation. As shown in table 1, the TCA-soluble radioactivity of the ACTH- and dbcAMP-treated cells is significantly lower than in the control conditions.

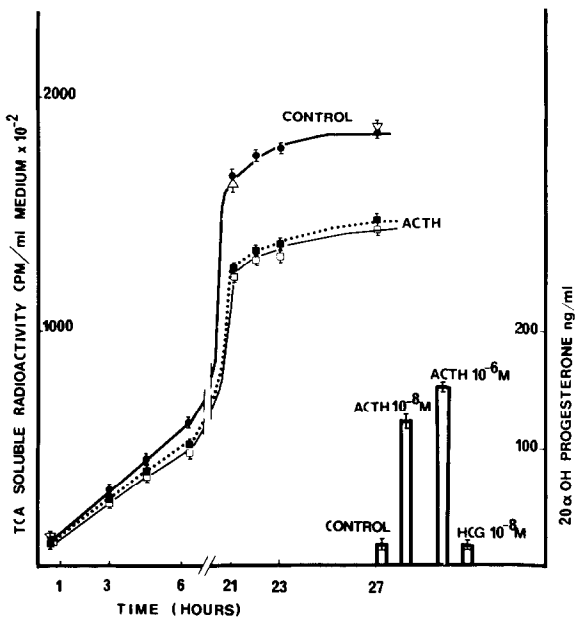

Fig.1. Effect of ACTH on the release of TCA-soluble radioactivity in the medium after the chase period (mean of triplicates ± SEM). (●—●) Control; (■···■) $ACTH_{1-24}$ 10^{-6} M; (□—□) $ACTH_{1-24}$ 10^{-8} M; (△) hCG 10^{-8} M. The bars represent the 20αOH progesterone ng/ml secreted in the medium at the end of the 27 h incubation (mean ± SEM of triplicate flasks).

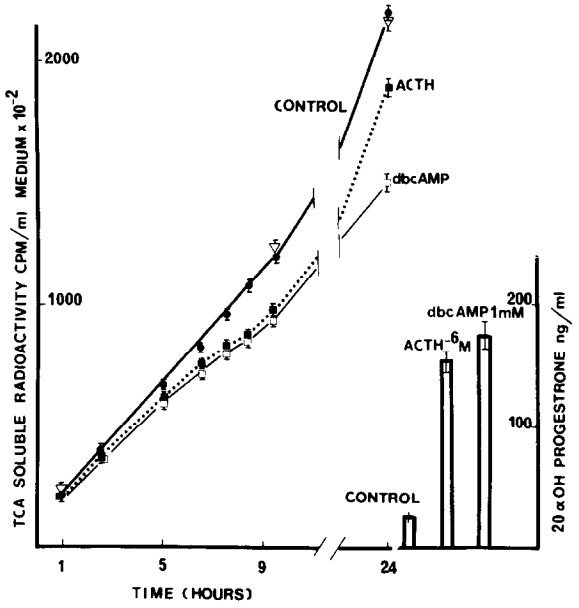

Fig.2. Effect of ACTH, dbcAMP, 20αOH progesterone on the release of TCA-soluble radioactivity in the medium after the chase period (mean of triplicates ± SEM). (●—●) Control; (■···■) $ACTH_{1-24}$ 10^{-6} M; (□—□) dbcAMP 1 mM; (△) 20αOH progesterone 100 ng/ml. The bars represent the 20αOH progesterone secreted in the medium at the end of 24 h incubation (mean ± SEM of triplicate flasks).

Table 1
TCA-soluble radioactivity in solubilized cells at the end of the sampling period described in fig.2 (mean ± SEM of triplicate flasks)

	cpm/μg proteins
Controls	1850 ± 20
ACTH 10^{-6} M	1620 ± 30
dbcAMP 1 mM	1530 ± 31
20αOH Progesterone 100 ng/ml	1860 ± 25

In these two experiments the labeling time was 15 h. The proteins whose degradation was inhibited by both ACTH and dbcAMP have a long turnover rate. Figure 3 shows that both ACTH and dbcAMP also inhibit the degradation of proteins with rapid rates of turnover. In this experiment the cells were incubated for 2 h with [^{3}H]leucine 20 μCi/ml, rinsed twice with the chase medium, and immediately incubated with ACTH and dbcAMP in the presence of 5 mM leucine (no chase period).

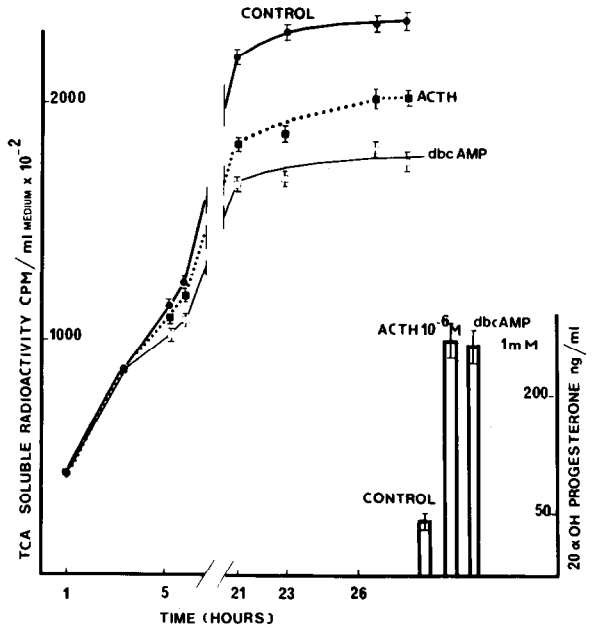

Fig.3. Effect of ACTH and dbcAMP on the release of TCA-soluble proteins after a two hours labeling period. Mean of triplicates ± SEM. (●–●) Control; (■···■) $ACTH_{1-24}$ 10^{-6} M; (□–□) dbcAMP 1 mM. The bars represent the 20αOH progesterone secreted in the medium at the end of the 28 h incubation (mean ± SEM of triplicate flasks).

The present results (fig.1–fig.3) show that ACTH and dbcAMP decrease the degradation of adrenal proteins with both long and short turnover rates. The quantitative significant difference observed between the action of ACTH and dbcAMP after 9 h incubation could be due in part to the fact that most of the hormone is degraded by the cells after 8 h incubation (unpublished data). Nevertheless our results suggest that cAMP formation could be an obligatory step for ACTH-inhibited adrenal proteolysis. To check this hypothesis the effects of two analogues of ACTH were investigated:

$ACTH_{11-24}$
: which is a pure antagonist of $ACTH_{1-24}$ concerning cAMP and steroid production, and which is able to bind to adrenal plasma membranes [12–14].

NPS-$ACTH_{1-24}$
: which binds to adrenal plasma membranes with the same affinity as $ACTH_{1-24}$ but which has a lower biological effect [12–14].

Figure 4 shows that cyclic AMP is probably not the only factor involved in the inhibition of adrenal protein degradation for two reasons:

1. $ACTH_{11-24}$ though less effective than $ACTH_{1-24}$ and NPS-$ACTH_{1-24}$ has a significant inhibitory effect.

2. On the other hand NPS-$ACTH_{1-24}$ was used at a higher concentration than $ACTH_{1-24}$. Nevertheless at this concentration the effect on steroidogenesis was less important than with $ACTH_{1-24}$, and the production of cyclic AMP is known to be less than 1% of maximum cyclic AMP production [14]. Despite a smaller biological effect on cAMP and steroid production, this analogue has a higher effect on protein degradation.

These results show that the C-terminal sequence of ACTH in addition to being responsible for the high

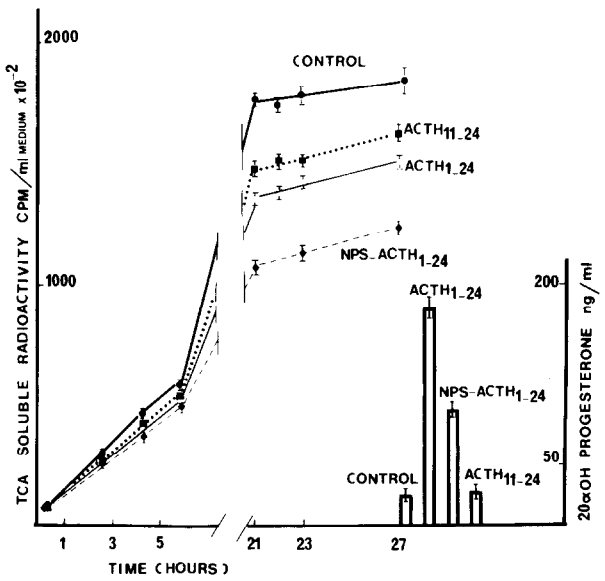

Fig.4. Effect of ACTH and its analogues on the release of TCA-soluble radioactivity. Cells were labelled for 15 h, rinsed and incubated in the chase medium as described in section 2, then incubated with the hormones. (●—●) Control; (■··■) $ACTH_{11-24}$ $2 \cdot 10^{-5}$ M; (□—□) $ACTH_{1-24}$ 10^{-6} M; (◆—◆) NPS-$ACTH_{1-24}$ $3 \cdot 10^{-5}$ M. The bars represent the 20αOH progesterone secreted in the medium at the end of 27 h incubation (mean ± SEM of triplicate flasks).

affinity of the hormone for its receptor, has some biological effects similar to those of $ACTH_{1-24}$.

This paper represents the first evidence that ACTH can directly inhibit adrenal protein degradation. Our results are in agreement with the work of Cannick and Villee [15] who showed indirectly that when ACTH has been chronically administered to rats the mean half-life of total adrenal proteins is significantly longer in ACTH-treated animals. This inhibition cannot completely account for the stimulation of protein synthesis we observed in these cell lines [4,5] for several reasons:

1. Stimulation of total protein synthesis of these cells is unaffected by cyclic AMP and by $ACTH_{11-24}$ or NPS-$ACTH_{1-24}$ [4].

2. Stimulation of protein synthesis by ACTH is maximal after 7 h incubation in the presence of ACTH, then decreases [4]. On the contrary our results show that inhibition of the proteolysis by ACTH reaches a plateau after 20 h incubation.

Studies are in progress in order to elucidate the mechanisms of this inhibitory effect and determine which proteins are protected from degradation by ACTH.

Acknowledgements

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